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Polyacrylamide nanosensor embedded with phosphate sensitive protein for detection of metabolic process in living cells

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Abstract: Phosphate is an essential nutrient for all plants. Accurate and rapid determination of phosphate is required to study phosphate homeostatis in living cell. Very few sensors reported display high selectivity and reliability. Our coworker Hong Gu has developed phosphate sensors (FLIPPi). The sensor was developed by fusing the phosphate binding protein (PiBP) into two variants of the green fluorescent protein. The sensor responded with a reversible, phosphate concentration-dependent decrease in Fluorescence Resonance Energy Transfer (FRET) efficiency.[2]

Fluorescent nanosensor particles, in which the fluorescent probes are embedded by cross-linked polymer in nanometer scale, have several advantages over direct loading of cells with fluorescent probes, which is a classical method for monitoring metabolic processes of living cells: The inert and biocompatible polymer matrix protects (a) the intracellular components from any toxicity of probes, and at the same time, (b) fluorescent protein from any potential interferences such as deactivation by protease; (c) the nanometer size minimizes the physical perturbation of the cell and the small size can provide fast response time for the sensor.[3]

A new polymer based phosphate nanosensor (PBPN) was made by embedding a phosphate sensor (FLIPPi-4μ) in polyacrylamide matrix through water-in-oil microemulsion polymerization, and purified by size exclusion chromatography, yielding particles of 50–80 nm in diameter. PBPN had the same binding and FRET properties as the pure FLIPPi-4μ protein. It kept 47% activity relative to that of FLIPPi-4μ, with ΔR_{max}=0.625 (R=YFP/CFP), and quantification range from 0.2 to 2 μM. PBPN provides a new tool for *in vivo* phosphate homeostasis study in the future.

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